A Research Note

PYRIDINE CATALYSIS OF ASCORBATE REDUCTION OF METMYOGLOBIN

INTRODUCTION

VARIOUS nitrogenous heterocyclic and aromatic compounds have been proposed as color-preserving agents for fresh and cured meats (Kendrick and Watt, 1969; Tarladgis, 1967; Bernholdt and Roschen, 1971; Hopkins and Sato, 1971; Coleman et al., 1949, 1951; Van Den Oord and DeVries, 1971; Dekker, 1958; Howard et al., 1973). Almost all of these authors include or recommend the use of ascorbic acid with the nitrogenous base. Coleman et al. (1951) modified their earlier patent application for the use of nicotinic acid (Coleman et al., 1949) to include the use of ascorbate in the process.

It seems unlikely that the mechanism of color formation could be the same for fresh and cooked cured meats, because in the former the pigments, myoglobin and hemoglobin, are in the native state, whereas in cooked cured meats the globin (protein) of the pigments has been denatured by heat. Howard et al. (1973) and Fox et al. (1974) have found that the protein must be denatured by sodium lauryl sulfate or by heat in order to form the pyridine hemochromes. Kendrick and Watts (1969) reported that a red hemochrome formed upon addition of nicotinic acid or nicotinamide to meat or solutions of reduced myoglobin, but the published spectra resemble the spectrum of oxymyoglobin more closely than the spectra of the pyridine hemochromes. While it is evident that nitrogenous bases produce a red color in cured meats by acting as heme ligands, their role in preserving the fresh meat color is obscure.

During our studies on pyridine derivatives as color-forming agents (Fox et al., 1974) we have observed that when these derivatives, plus a reductant, were mixed with bovine metmyoglobin only the reduced form of the pigment was formed; i.e., myoglobin in nitrogen or with sodium dithionite, or oxymyoglobin in air. We found no evidence of pyridine-heme interaction but observed that the rate of reduction varied with different pyridine derivatives.

EXPERIMENTAL

METMYOGLOBIN (Nicholas and Fox, 1969), 0.05 mM in μ = 0.050 acetate buffer (pH 5.5), was reduced with 20 mM ascorbate in the presence of 15 mM pyridine derivatives at 20°C. The reaction was followed by repetitive scanning in a Cary 14 spectrophotometer from 600–450 nm, which range includes the absorption maxima of metmyoglobin (502 nm) and oxymyoglobin (543 and 580 nm).

Table 1—Reduction of metmyoglobin to oxymyoglobin by ascorbate in the presence of substituted pyridines

Pyridine substituent	k_{1st}, min^{-1}
Control	0.0387
Pyridine	1.37
3-CH_3	0.95
4-CH_3	0.22
3-COOH	3.02
3-$COCH_3$	0.328
4-$COCH_3$	0.052
3-$CONH_2$	0.081
2-COOMe	0.040
3-COOMe	0.83
4-COOMe	0.060
Coefficient of variation, %	7.3

RESULTS & DISCUSSION

THE FIRST-ORDER rate constants for the reduction of metmyoglobin in air by ascorbate in the presence of substituted pyridines (Table 1), show a catalytic effect of the derivatives. The experiment was repeated with 20 mM cysteine as the reductant and the same pyridine derivatives but the rate constant was not increased significantly. We propose that the observed catalysis explains the color improvement in fresh meats reported by the aforementioned authors and patentees. In corroboration of this proposal, we note that the catalytic effect of nicotinic acid was the greatest, while the effect of nicotinamide was barely greater than that of the control. This finding supports Kendrick and Watts' (1969) report that the former was better than the latter in preserving fresh meat color. When exposed to air the heme pigments of meat are in a constant cycle of oxidation and reduction and any compound which accelerates the reduction part of the cycle will improve red color, since only the reduced pigment can form oxymyoglobin. In view of the effectiveness of the nonphysiological pyridine derivatives (pyridine, 3- and 4-methylpyridine, all 4-position substituted pyridines) the effect appears to be a chemical catalysis of the ascorbate reduction of the heme pigments, and not a biochemical or physiological response. In all reactions the 3-derivatives were more effective than the corresponding 4-derivatives. The 3- and 4-derivatives also tended to form differently colored hemochromes (Fox et al., 1974), but we can not, as yet, completely explain either phenomenon.

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